Pergamon

Tetrahedron Letters 43 (2002) 1665–1668

TETRAHEDRON LETTERS

Structure and absolute configuration of acetosellin, a new polyketide from a phytotoxic strain of *Cercosporella acetosella*†

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Received 30 November 2001; revised 10 January 2002; accepted 11 January 2002

Abstract—A new yellow pigment, designated acetosellin **1**, was isolated from the mycelium of *Cercosporella acetosella* as a reduced azaphilone metabolite. The structure of **1** was determined by spectroscopic investigations and chemical evidence. Compound **1** possesses a novel carbon skeleton that of a naphtopyrane derivative, linked to an extensively conjugated chain. The absolute configuration was determined by NOE experiments and CD correlations.

In the course of a program aimed to identify new bioactive compounds from phytopathogenic fungi,[2] the mitosporic species *Cercosporella acetosella* Ell.[3] was isolated as a pathogen from leaf spots of the cosmopolitan weed *Rumex acetosella* L. This paper deals with the isolation, structure elucidation and biological activities of acetosellin (**1**), a new pigment isolated from large-scale cultures of the fungus.

The crude extract was recovered with EtOAc from potato-dextrose-agar (PDA) cultures of the strain in Roux flasks at 7 days of growth and purified by silica gel chromatography eluted with a stepwise mixture of CH_2Cl_2–MeOH, followed by repeated PLC.

Acetosellin (**1**) was isolated as a yellow solid having mp 155–160°C, $[\alpha]_D = +283$ (*c* 0.1, MeOH); the EIMS and CIMS of **1** showed a molecular ion at *m*/*z* 394, corresponding to $C_{23}H_{22}O_6$. The IR spectrum exhibited absorptions at 3400 cm^{-1}, 1750 and 1690 cm^{-1} suggesting the presence of hydroxy, unsaturated lactone and carbonyl groups; the UV spectrum showed λ_{max} 210, 230sh, 245sh, and 340 nm (ε 20800, 16600, 15900, 22700).

Acetylation of **1** afforded the diacetyl derivative **2**, mp 105–108°C, $[\alpha]_D = +200$ (*c* 0.2, $CHCl_3$) indicating the presence in the molecule of two OH groups; **1** reacted with diazomethane in Et_2O to give the monomethyl ether **3**, supporting that one hydroxy function is located on an aromatic ring. In addition to the resonances due to the two acetate groups, the ^{13}C NMR spectrum of **2** contained 23 signals. The sp^2 signals were assigned to the above-mentioned C-9 and C-11 carbonyl carbons and to the carbons of a pentasubstituted aromatic ring ($^1J_{C,H} = 163.5$ Hz) and of three disubstituted ($^1J_{C,H} =$ 149–154 Hz) and one tetrasubstituted double bonds. The sp^3 signals were assigned to two methyl, to three methylene (two of them oxygen bearing), to one oxygen bearing methine and to one oxygen bearing quaternary carbons.

The ^1H NMR spectrum (Table 1) extended the above evidence through the appearance of one tertiary methyl group (H_3-16) which was allocated at the sole quaternary sp^3 carbon C-8 (its protons presented a long-range C,H coupling of 4.5 Hz with C-8), of one aromatic proton (H-14), of one isolated AB system (H_2-1) and of two sequences such as ′–C(1′)H=C(2′)H-C(3′)H=C(4′)H-C(5′)H=C(6′)HMe and –C(4)H_2–C(3)HOR–C(17)H_2-OAc in which the olefinic protons are all E, as evidenced by the $^3J_{H,H} = 14.9$–15.2 Hz, and the OAc group is linked at C-17 as H_2-17 underwent a downfield shift of ~0.6 ppm by acetylation. NOE experiments carried out on compounds **2** and **3** (see Data) indicated that the aromatic OH group and the hexatriene chain are both *ortho* positioned with respect to H-14.

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† Secondary fungal metabolites: part 61; for part 60, see Ref. 1.

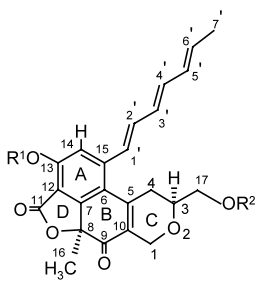
0040-4039/02/$ - see front matter
PII: S0040-4039(02)00091-6

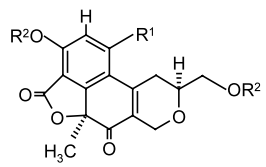
Table 1. ^{1}H NMR chemical shifts for compounds **2** and **7** in acetone-d_6

Proton	**2** (δ_H, ppm)		J(H,H) (Hz)	Proton	**7** (δ_H, ppm)		J(H,H) (Hz)
1a	4.71	ddd	17.5, 3.1, 1.3	1	5.02,4.78	br d	17.2
1b	4.44	ddd	17.5, 3.9, 3.1	3	3.51	m	
3	3.98	dddd	10.2, 6.0, 3.7, 3.1	4	3.16, 3.02	m	
4a	3.00	dddd	17.6, 10.2, 3.9, 3.1	11, 13	7.17, 6.95	d	1.8
4b	2.88	dddd	17.6, 3.1, 3.1, 1.3	15	2.41	s	
14	7.38	s		16	3.70, 3.68	m	
16	1.91	s		1′	7.32	br d	15.3
17a	4.30	dd	11.9, 3.7	2′	6.50	m	
17b	4.23	dd	11.9, 6.0	3′	6.36	m	
1′	7.26	br dd	15.2	4′	6.40	m	
2′	6.94	br dd	15.2, 10.2	5′	6.19	m	
3′	6.45	br dd	15.1, 10.2	6′	5.77	dq	15.0, 7.0
4′	6.53	br dd	15.1, 10.2	7′	1.78	dd	7.0, 1.8
5′	6.22	ddq	14.9, 10.2, 1.7	OH-9, -12	9.46, 7.65	br s	
6′	5.90	dq	14.9, 6.9	OH-16	3.85	br signal	
7′	1.80	dd	6.9, 1.7				
13-OAc	2.35	s					
17-OAc	2.06	s					

On catalytic hydrogenation with 10% palladium on carbon, **1** gave the hexahydroderivative **4**, M^+, m/z 400 which, in turn, afforded the expected diacetate **5**; the ^{1}H NMR spectrum revealed that the reduction occurred only at the three double bonds of the side chain. The reaction of compound **1** with OsO_4 and KIO_4, afforded the derivative **6**, which presented an aldehydic group in place of the unsaturated chain.

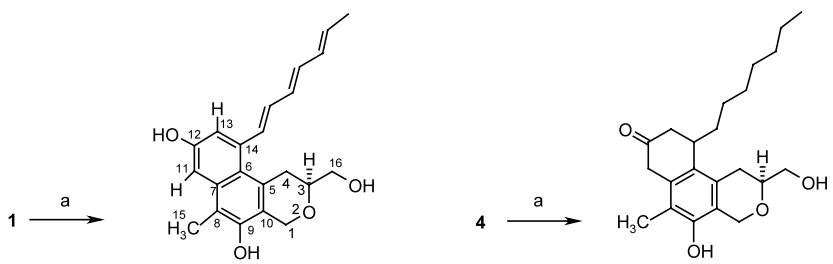
Further information on the structure of acetosellin followed from the reaction of **1** with Zn and H_2SO_4; in fact, the isolated compound **7**, which analyzed for $C_{22}H_{24}O_4$, presented no carbonyl bands in the IR spectrum and showed two additional protons in the ^{1}H NMR spectrum (Table 1) attributable to one aromatic OH group and to one aromatic proton *meta* positioned with respect to H-13 ($^4J_{H,H}$ = 1.8 Hz); moreover, the





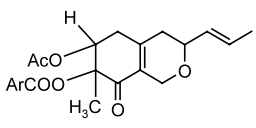
1 $R^1 = R^2 = H$
2 $R^1 = R^2 = Ac$
3 $R^1 = Me$; $R^2 = H$

4 $R^1 = -(CH_2)_6$-Me; $R^2 = H$
5 $R^1 = -(CH_2)_6$-Me; $R^2 = Ac$
6 $R^1 = CHO$; $R^2 = H$



7 **8**

a) Zn, MeOH-H_2SO_4 (95:5), 0.5 hour.



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15-methyl protons resonated at 2.41 ppm suggesting that the C(15)H_3 group is located on an aromatic ring. All these findings can be explained by the acidic opening of the lactone ring D followed by reductive decarboxylation and aromatization of the ring B to gave **7**. The same reaction made on **4** gave in good yield the major product **8** which presented the partial reduction of the aromatic ring A too; compound **8** is homogeneous but the stereochemistry of the new C-14 chiral center was not determined.

The remaining $C_4H_8O_2$ fragment of compound **2** must be part of the dihydropyran ring C in which the C(4)H_2 group is linked at C-5, since the 4-H_2 protons presented a mutual NOE with H-1′ (see Data). The similarity of the aliphatic portion of acetosellin with that of wortmin **9**, a metabolite isolated by us in *Penicillium wortmanni*,[4] justified these assignments.

Finally, the mutual NOEs observed between H-3 and H_3-16 in compound **2** indicated that these protons are on the same side of the molecule permitting us to assign the relative configuration of C-3 and C-8.

The structure **1** allows us to include acetosellin among the class of azaphilone metabolites, produced by fungi belonging to different genera: *Aspergillus*, *Monascus*, *Penicillium* and *Chaetomium*. Among azaphilones bearing a five-membered lactone ring, two types of junction are known: the linear type as in rotiorin, monascorubrin and monascoflavin, and the angular type as in rubrorotiorin, deflectins and chaetoviridins;[5] acetosellin **1** evidently belongs to the first type.

The absolute configuration at C-8 of azaphilones so far known was established from CD curves;[6] the sign of the Cotton effect at the longest wavelength depends on the configuration at the C-8 position. The CD of **1** ($\Delta\varepsilon_{340}=-10$) clearly showed the (*S*) configuration at C-8 and the absolute configuration of acetosellin **1** was consequently concluded to be as shown in **1**. Moreover, the structure **1** is the most probable on the basis of biogenetic arguments, owing to the close similarity to others members of the azaphilone group.[7] Acetosellin is as an interesting new example having a low oxidation level in the rings B and C, it appears as a polyketide composed of a main chain (4 units) starting from the side chain at C_{17} and ending at C_{11} with two subsidiary chains attached at the C-7 (2 units) and C-12 (5 units).

Acetosellin (**1**) was tested for biological activity: it did not show any antifungal and antitumoral activity and had a weak lethal activity for *Micrococcus luteus* and *Saccharomyces cerevisiae*. Acetosellin inhibited *Lepidium sativum* and *Zea mais* in the root elongation assays[8] at $6.4\cdot 10^{-4}$ M, but could not be reisolated from the test solution at the end of the trial.

Physical and spectroscopic data of the compounds

1: Anal. C, 69.84%; H, 5.69%, calcd for $C_{23}H_{22}O_6$, C, 70.04%; H, 5.63%; CD (MeOH, *c* mg/cm^3 0.1): 216, 245, 271 and 340 ($\Delta\varepsilon$ +10, −1, +4,5, −10); ^{1}H NMR acetone-d_6 (δ/ppm): 9.90 (1H, br signal, OH-13), 7.21 (1H, br d, $J=15.3$ Hz, H-1′), 7.03 (1H, s, H-14), 6.78 (1H, br dd, $J=15.3$ and 10.1 Hz, H-2′), 6.47 (1H, br dd, $J=14.9$ and 10.0 Hz, H-4′), 6.42 (1H, br dd, $J=14.9$ and 10.1 Hz, H-3′), 6.19 (1H, dd q, $J=15.0$, 10.0 and 1.8 Hz, H-5′), 5.87 (1H, dq, $J=15.0$ and 7.0 Hz, H-6′), 4.68 1H, br dd, $J=17.2$ and 3.1 Hz, H-1a), 4.34 (1H, ddd, $J=17.2$, 3.8 and 3.0 Hz, H-1b), 3.95 (1H, br signal, OH-17), 3.8–3.6 (3H, m, H_2-17 and H-3), 2.93 (1H, m, H-4a), 2.78 (1H, br ddd, $J=17.6$, 3.1, and 3.0 Hz, H-4b, 1.84 (3H, s, H_3-16), 1.79 (3H, dd, $J=7.0$ and 1.8 Hz, H_3-7′). ^{13}C DMSO-d_6 (δ/ppm): 196.71 (S, C-9), 164.21 (S, C-11), 157.78, 146.84, 146.50, 144.94 (4×S, C-5, -7, -13, -15), 135.44, 134.21, 131.88. 131.50, 130.51, 124.24 (6×D, C-1′, -2′, -3′, -4′, -5′, -6′), 130.51, 117.72, 109.03 (3×s, C-6, -10, -12), 119.19 (D, C-14), 84.79 (S, C-8), 74.21 (D, C-3), 63.77 (2×T, C-1, -17), 32.04 (T, C-4), 28.78 (Q, C-16), 18.18 (Q, C-7′).

2: M$^+$, *m*/*z* 478; anal. C, 67.61%; H, 5.39%, calcd for $C_{27}H_{26}O_8$, C, 67.77%; H, 5.48%; ^{13}C NMR DMSO-d_6 (δ/ppm): 194.47 (S, C-9), 170.96 and 168.32 (2×S, 2×$CH_3\underline{C}O_2$), 164.65 (S, C-11), 154.62, 147.46, 144.71, 142.68 (4×S, C-5, -7, -13, -15), 137.88, 137.35, 133.80. 131.34, 129.02, 127.04 (6×D, C-1′, -2′, -3′, -4′, -5′, -6′), 130.41, 122.57, 115.48 (3×S, C-6, -10, -12), 123.67 (Dd, $^1J=163.5$ and $^3J=5.5$ Hz, C-14), 85.28 (Sq, $^2J=4.5$ Hz, C-8), 71.49 (D, C-3), 65.79 and 64.81 (2×T, C-1, -17), 32.43 (T, C-4), 27.51 (Q, C-16), 20.81 and 20.58 (2×Q, 2×$\underline{C}H_3CO_2$), 18.51 (Q, C-7′). Selected NOE experiments ($CDCl_3$): {H-3} enhanced H-1b (3.5%), H_3-16 (0.5%), H_2-17 (2%); {H-4a} enhanced H-1′ (5%); {H-4b} enhanced H-3 (1.5%) and H-1′ (2.5%); {H-14} enhanced H-2′ (14%); {H_3-16} enhanced H-1b (1%) and H-3 (1%) {H-1′} enhanced H_2-4 (1.5%) and H-3′ (3.5%).

3: mp 142–145°C; M$^+$, *m*/*z* 408. ^{1}H NMR acetone-d_6 (δ/ppm): 7.26 (1H, br d, $J=15.3$ Hz, H-1′), 7.16 (1H, s, H-14), 6.88 (1H, m, H-2′), 6.46 (1H, m, H-4′), 6.40 (1H, m, H-3′), 6.21 (1H, m, H-5′), 5.89 (1H, m, H-6′), 4.68 and 4.36 (2H, br d, $J=17.2$ Hz, H_2-1), 4.05 (3H, s, 13-OMe), 4.00 (1H, br signal, OH-17), 3.8–3.6 (3H, m, H_2-17 and H-3), 2.90 and 2.80 (2H, m, H_2-4), 1.85 (3H, s, H_3-16), 1.80 (3H, dd, $J=7.0$ and 1.8 Hz, H_3-7′). {13-OMe} enhanced H-14(15%).

4: mp 92–95°C; $[\alpha]_D=335$ (*c* 0.2, MeOH); UV λ_{max} 250sh and 320 (ε 10600 and 8900); ^{1}H NMR $CDCl_3$ (δ/ppm): 6.73 (1H, s, H-14), 4.86 (1H, ddd, $J=17.5$, 2.9 and 1.1 Hz, H-1a), 4.40 (1H, ddd, $J=17.5$, 3.8 and 3.1 Hz, H-1b), 3.95–3.65 (3H, m, H_2-17 and H-3), 3.15–2.55 (4H, m, H_2-4 and -1′), 2.50 (1H, br signals, OH-17), 1.88 (3H, s, H_3-16), 1.7–1.1 (10H, m, H_2-2′, -3′, -4′, -5′, -6′), 0.90 (3H, t, $J=6.0$ Hz, H_3-7′).

5: oil, M$^+$, *m*/*z* 484; ^{1}H NMR $CDCl_3$ (δ/ppm): 7.01 (1H, s, H-14), 4.85 and 4.42 (2H, br d, $J=17.5$ Hz, H_2-1), 4.33 and 4.30 (2H, m, H_2-17), 3.90 (1H, m, H-3), 3.15–2.55 (4H, m, H_2-4 and -1′), 2.37 (3H, s, 13-OAc), 2.13 (3H, s, 17-OAc), 1.87 (3H, s, H_3-16), 1.8–1.1 (10H, m, -2′, -3′, -4′,- 5′, -6′), 0.89 (3H, t, $J=6.0$ Hz, H_3-7′).

6: **1**+dioxane/OsO_4/KIO_4/rt 10 min; M^+, m/z 330, 312 (M^+-18) (312.0710 calcd for $C_{17}H_{12}O_6$ 312.0634); λ_{max} 320 nm (ε 8900). ^1H NMR acetone-d_6 (δ/ppm): 10.72 (1H, s, H-1′), 7.37 (1H, s, H-14), 5.00 (1H, br signal, OH-17), 4.74 and 4.40 (2H, br d, $J=17.5$ Hz, H_2-1), 4.0–3.4 (3H, m, H_2-17 and H-3), 2.95 and 2.90 (2H, m, H_2-4), 1.90 (3H, s, H_3-16).

7: M^+, m/z 352; anal. C, 74.78%; H, 6.67%, calcd for $C_{22}H_{24}O_4$, C, 74.97%; H, 6.86%; the ^1H NMR spectrum is in Table 1.

8: oil, M^+, m/z 360(20%), 261 (M^+-99) (100), 233(38). ^1H NMR $CDCl_3$ (δ/ppm): 5.10 (1H, br signal, OH-9), 5.05 and 4.72 (2H, br d, $J=15.4$ Hz, H_2-1), 3.9–3.7 (3H, m, H_2-16 and H-3), 3.54 and 3.39 (2H, d, $J=21.7$ Hz, H_2-11), 3.25 (1H, m, H-14), 2.73 (1H, dd, $J=15.2$ and 2.6 Hz, H-13a), 2.69 and 2.60 (2H, m, H_2-4), 2.58 (1H, dd, $J=15.2$ and 5.5 Hz, H-13b), 2.50 (1H, br signal, OH-16), 2.08 (3H, s, H_3-15), 1.5–1.1 (12 H, m, H_2 -1′ -6′), 0.86 (3H, t, $J=6.2$ Hz, H_3-7′). ^{13}C NMR $CDCl_3$ (δ/ppm): 210.65 (S, C-12), 148.03, 131.38, 130.30, 128.33, 120.71, 118.4 (6×S, ArC), 75.00 (D, C-3), 65.79 and 64.93 (2×T, C-1,-16), 43.23, 41.42, 34.78, 31.82, 29.50, 29.14, 27.24, 26.80, 22.63 (9×T, C-4, -11, -13, -1′, -2′, -3′, -4′, -5′, -6′), 34.86 (D, C-14), 14.09 (Q, C-7′), 11.02 (Q, C-15). Selected NOE experiments ($CDCl_3$): {H_2-11} enhanced H_3-15 (0.5%); {H_3-15} enhanced H_2-11 (3.5%).

References

1. Arnone, A.; Assante, G.; Nasini, G.; Strada, S.; Vercesi, A. *J. Nat. Prod.* **2002**, in press.
2. Arnone, A.; Nasini, G.; Vajna de Pava, O. *Phytochemistry* **1998**, *48*, 507–510 and references cited therein.
3. Strain IPV 166, belonging to the Mycological Collection of 'Istituto di Patologia Vegetale, Università di Milano'.
4. Merlini, L.; Mondelli, R.; Nasini, G.; Hesse, M. *Helv. Chim. Acta* **1973**, *56*, 232–239.
5. Takahashi, M.; Koyama, K.; Netori, S. *Chem. Pharm. Bull.* **1990**, *38*, 625–628.
6. (a) Steyn, P. S.; Vleggar, R. *J. Chem. Soc., Perkin 1* **1976**, 204–206; (b) Hashimoto, T.; Asakawa, Y. *Heterocycles* **1998**, *47*, 1067–1170.
7. Thomas, R. *Chembiochem* **2001**, *2*, 612–627.
8. Arnone, A.; Assante, G.; Nasini, G.; Vajna de Pava, O. *Phytochemistry* **1990**, *29*, 613–616.